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The validity of *Pelophylax chosenicus* (Okada, 1931) and *P. hubeiensis* (Fei & Ye, 1982) (Amphibia, Ranidae)

Sheng-Bo Zhou[1], Qiu-Yi Zhang[1], Zi-Qiang Hu[1], Zu-Yao Xia[2], Qing Miao[1], Ping Guan[1], Jing-Song Shi[3,4]

1 *Key Laboratory of Global Changes and Biological Invasions, Bioscience and Technology College, Shenyang Agricultural University, Shenyang 110866, China*
2 *Department of Evolution, Ecology & Biodiversity University of California, Davis Davis, CA 95616, USA*
3 *Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*
4 *Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China*

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Corresponding author: Ping Guan (492508453@qq.com)

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Abstract

Pelophylax plancyi (Lataste, 1880), *Pelophylax chosenicus* (Okada, 1931) and *Pelophylax hubeiensis* (Fei & Ye, 1982) were described chronologically from East Asia. The three species have similar morphological and molecular characteristics, but the taxonomic relationships amongst them have long been ambiguous. To deal with this taxonomic issue, we examined newly-obtained topotypic specimens of *P. plancyi*, *P. chosenicus* and *P. hubeiensis* for morphological comparison. Furthermore, we investigated the phylogeny of pond frogs in Eurasia by Bayesian Inference and Maximum Likelihood analyses of a fragment consisting of mitochondrial DNA gene 16s and provided a molecular phylogeny of the genus *Pelophylax*. There were no morphological and molecular differences between *P. plancyi* and *P. chosenicus*, but both morphological and molecular differences between *P. hubeiensis* and *P. plancyi*. Hence, we conclude that *P. chosenicus* is a junior synonym of *P. plancyi* and *P. hubeiensis* is a separate species from *P. plancyi*.

Key Words

amphibians, morphology, phylogeny, synonym, taxonomy

Introduction

The pond frogs of the genus *Pelophylax* Fitzinger, 1843 originated from the western Palearctic (Pyron 2014) and are continuously distributed across the Palearctic (Lymberakis et al. 2007; Frost 2022). Including the record of *Pelophylax chosenicus* (Okada, 1931) lately, seven of them were reported to be distributed in China. (AmphibiaChina 2022; AmphibiaWeb 2022; Zhou et al. 2022). Even though the evolutionary relationships on these frogs are settled, partial members still have questionable taxonomic status in this genus because of substantial intraspecific morphological variation and high levels of interspecific morphological similarity, such as the validities of *P. chosenicus* (Shannon 1956; Dubois and Ohler 1994; Amal et al. 2017; Dufresnes and Litvinchuk 2022) and *P. hubeiensis* (Fei & Ye, 1982) (Mou and Zhao 1992; Zhao and Adler 1993; Dufresnes and Litvinchuk 2022).

Based on specimens collected from Seoul, South Korea, Okada (1927) described a subspecies *Rana nigromaculata coreana* (Okada 1927, 1928) and subsequently changed the scientific name to *R. n. chosenica* (Okada 1931). In 1956, Shannon modified *R. n. chosenica* to *R. plancyi chosenica* as a subspecies of *R. plancyi* (*sensu P. plancyi* (Lataste, 1880) in this study) (Shannon 1956). Kuramoto (1983) identified it as a separate species, *R. chosenica* (sensu *P. chosenicus* in this study), which was later endorsed by Dubois (1986, 1992), but Dubois and Ohler (1994) further identified *R. chosenica*

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as a subspecies of *R. plancyi*. Amphibian Species of the World (Frost 2022) placed *P. chosenicus* as a valid species in the genus *Pelophylax*. Fei and Ye (1982) described *Rana hubeiensis* (sensu *P. hubeiensis* in this study), based on specimens from Hubei and Anhui Provinces, but Mou and Zhao (1992) suggested, based on identical call parameters, that *P. hubeiensis* is a junior synonym of *P. plancyi*. Based on the obvious morphological differences (e.g. male with silent sac), Fei et al. (2009) still regarded it as a valid species. Dufresnes and Litvinchuk (2022) placed both *P. chosenicus* and *P. hubeiensis* into the synonymy of *P. plancyi*, based on low genetic distances. The two species are pending a more detailed review of the taxonomic issue (Frost 2022).

From 2021 to 2022, we collected a series of topotypic specimens of *P. plancyi*, *P. chosenicus* and *P. hubeiensis* (Fig. 1). The combined evidence from morphology and molecular phylogeny of these specimens demonstrated the distinct specific level of *P. hubeiensis* that is distant from *P. plancyi*, while indicating the homogeneity between *P. chosenicus* and *P. plancyi*. Thus, we suggest elevating *P. hubeiensis* as a separate species from *P. plancyi* and placing *P. chosenicus* to be a secondary synonym of *P. plancyi*.

Materials and methods

Specimens and sampling

We collected six specimens (three adult males and three adult females) of *Pelophylax chosenicus* from multiple localities of Seoul, South Korea and Shenyang, Dandong, China, six specimens (three adult males and three adult females) of *P. plancyi* were collected from four localities in China (Fig. 1) and six specimens (three adult males and three adult females) of *P. hubeiensis* were collected from Lichuan and Wuhan, China, including specimens from the vicinity of the type locality of the three species (detailed collection information presented in the Taxonomic account below). The specimens collected from South Korea were released after morphological data measuring and Non-Disruptive DNA Sampling. The DNA- sampling came from oral throat swab sampling. The specimens collected from China were fixed in 10% formalin and then stored in 75% ethanol. Liver tissue samples were collected from individual specimens before formalin fixation and preserved in 95% ethanol for subsequent molecular analyses. Specimens and tissue samples are preserved at Shenyang Agricultural University (**SYAU**).

Morphological comparison and analysis

Linear measurements were performed on all the adult specimens, using a Vernier caliper with a precision of 0.1 mm, with the following abbreviations: **SVL** (snout-vent length, from the tip of snout to vent); **HL** (head length, from posterior corner of the mandible to tip of snout); **HW** (head width, the greatest cranial width); **SL** (snout length, from the tip of snout to the anterior corner of the eye); **INS** (internasal space, the distance between the two nostrils); **IOS** (interorbital distance, the minimal distance between upper eyelids); **ED** (horizontal eye diameter); **LAHL** (length of lower arm and hand, from the tip of finger III to the elbow joint); **TD** (horizontal tympanic diameter); **LAD** (diameter of the lower arm); **HLL** (hind limb length, from the tip

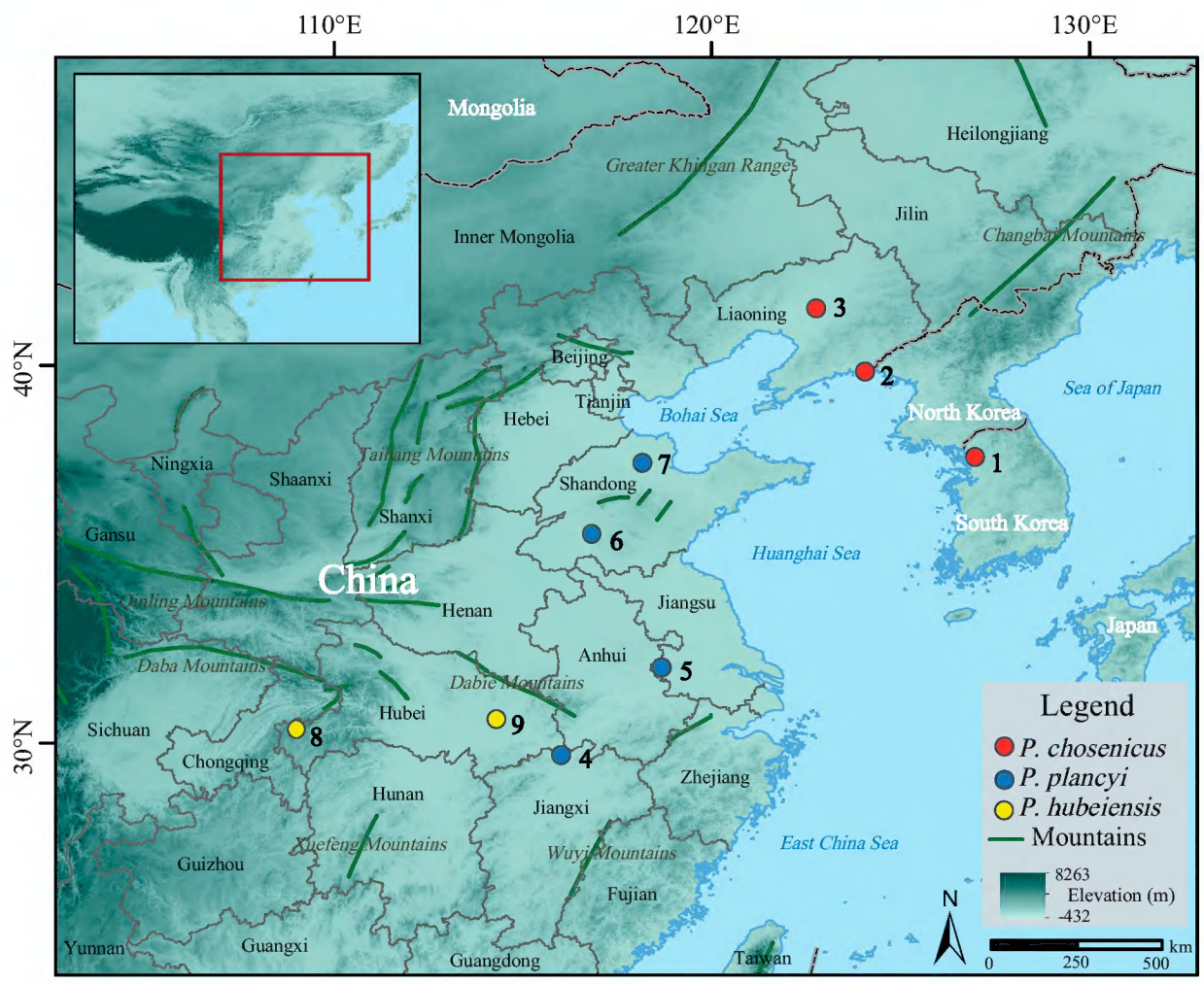

Figure 1. The collection localities of *Pelophylax chosenicus* (marked with red circles), *P. plancyi* (marked with blue circles) and *P. hubeiensis* (marked with yellow circles) in this study. (1) Liulin Wetland, Seoul, South Korea; (type locality of *P. chosenicus*); (2) Donggang District, Dandong, China; (3) Puhe Park, Shenyang, China; (4) Nanshan Park, Jiujiang, China (type locality of *P. plancyi*); (5) Yuzui Park, Nanjing, China; (6) Si River, Jining, China; (7) Bailuhu Park, Binzhou, China; (8) Qing River, Lichuan, China (type locality of *P. hubeiensis*); (9) Jiefang Park, Wuhan, China.

of toe IV to groin); **TL** (tibia length); **TW** (tibia width, the greatest width of tibia); **FL** (foot length, from the proximal end of the inner metatarsal tubercle to the tip of toe IV); and **IMT** (internal metatarsal tubercle, the length of internal metatarsal tubercle). The morphology comparisons are according to Zhou et al. (2022) for *Pelophylax mongolius* (Schmidt 1925) and Fei et al. (2009) for *P. nigromaculatus* (Hallowell, 1861), *P. fukienensis* (Pope, 1929) and *P. terentievi* (Mezhzherin, 1992). The toe webbing formula followed the protocol described by Savage (1975).

Measurement data were used for principal component analysis (PCA) on the morphometric differences amongst *P. plancyi*, *P. chosenicus* and *P. hubeiensis*. Statistical analyses were carried out by using the "prcomp" package in R 4.1.1 (Zhang et al. 2022).

Phylogenetic analyses

Genomic DNA was extracted by Qiaprep Spin Miniprep kits (QiaGen) and a 508 bp mitochondrial genome fragment of *16S* ribosomal RNA (*16S*) using primers L3975 and H4551 (Simon et al. 1994) was specifically amplified for this study. The standard PCR protocol was performed in a 20 µl reaction with at least 20 ng of template DNA and 10 pmol of primers. PCR conditions consisted of an initial denaturation for 5 min at 95 °C, followed by 25 cycles: denaturation at 95 °C for 30 sec, annealing at 54 °C for 60 sec, elongation at 72 °C for 90 sec and then finalised with an extension step of 10 min at 72 °C. Sequencing was conducted by Nanchang Kechang Biotech Co., Ltd. New sequences are uploaded to GenBank (Table 1).

For phylogenetic comparisons, corresponding sequences of 11 recognised species of the genus *Pelophylax* and one outgroup (*Rana taihangensis* Chen, 2022) were obtained from GenBank (Table 1). Sequences were assembled and aligned using MEGA7 (Tamura et al. 2013) with default settings and were further revised manually when necessary. Phylogenetic trees were estimated with Maximum Likelihood (ML) using RAxML NG v.0.9.0. (Kozlov et al. 2019) and Bayesian Inference (BI) using MrBayes 3.1.2 (Ronquist et al. 2012). We used JModelTest 2 (Guindon and Gascuel 2003; Darriba et al. 2012) to infer the model of sequence evolution that best fits our

Table 1. Species, voucher museum numbers, sample localities and GenBank accession numbers for 16S rRNA of *Pelophylax* species used in the phylogenetic analyses.

Taxa	Voucher	Country: localities	16S rRNA accession number	Reference
Pelophylax chosenicus	HGSE 01	South Korea: Seoul	OQ708390	This study
	HGSE 02		OQ708391	
	SYAU BAA000061	China: Liaoning: Dandong	OQ708385	
	SYAU BAA000062		OQ708386	
	SYAU BAA000046	China: Liaoning: Shenyang	OQ708387	
	SYAU BAA000016		OL752662	Zhou et al. (2022)
	SYAU BAA000017		OL752663	
	SYAU BAA000018		OL752664	
	NIBRAM0000000038	South Korea: Chungcheongbuk-do: Goesan-gun	JQ815307	Jeong et al. (2013)
	NIBRAM0000100371	South Korea: Chungcheongnam-do: Taean-gun	JQ815308	
	MMS176	South Korea	EU386945	Direct submission by Min et al. (2016)
	MMS179		EU386932	
	MMS533		EU386947	
	MMS431		EU386935	
	MMS446		EU386958	
	MMS524		EU386959	
	MMS531		EU386943	
	MMS189		EU386944	
	MMS171		EU386946	
	MMS102		EU386914	
	MMS510		EU386908	
	MMS523		EU386941	
	–		JF730436	Ryu and Hwang (2011)
P. plancyi	SYAU BAA000035	China: Jiangxi: Jiujiang	OQ708392	This study
	SYAU BAA000072		OQ708393	
	SYAU BAA000073		OQ708394	
	SYAU BAA000074	China: Jiangsu: Nanjing	OQ708395	
	SYAU BAA000075	China: Shandong: Binzhou	OQ708396	
	SYAU BAA000077	China: Shandong: Jining	OQ708397	
P. hubeiensis	SYAU BAA000052	China: Hubei: Lichuan	OQ708388	
	SYAU BAA000051	China: Hubei: Wuhan	OQ708389	
	–	China: Anhui: Huoqiu	AF315137	Direct submission by Jiang and Zhao (2005)
P. nigromaculatus	–	Japan	AB043889	Masayuki et al. (2001)
P. mongolius	SYAU BAA000001	China: Ningxia: Guyuan	OL752643	Zhou et al. (2022)
	SYAU BAA000030	China: Inner mongolia: Baotou	ON693246	
P. porosus	Pp2	Japan: Aichi	LC389210	Tokumoto et al. (2019)
P. cretensis	NHMC 80.2.46.18	Greece	DQ474204	Lymberakis et al. (2007)
P. epeiroticus	NHMC 80.2.109.4		DQ474207	
P. kurtmuelleri	NHMC 80.2.11.12		DQ474228	
P. bedriagae	NHMC.80.2.99.24		DQ474193	
P. cerigensis	NHMC.80.2.110.5		DQ474196	
Rana taihangensis	SYAU BAA000025	China: Ningxia: Guyuan	OQ708398	This study

16S sequences. The model HKY + I + G was selected as the best-fitted model for BI testing and HKY + F + R2 was selected as the best-fitted model for ML. All searches consisted of three heated chains and a single cold chain. Three independent iterations, each comprising two runs of 100,000,000 generations, were performed, sampling every 10,000 generations and parameter estimates were plotted against generation. The first 25% of the samples were discarded as burn-in, resulting in a potential scale reduction factor (PSRF) of < 0.005. Maximum likelihood analysis is completed through 1000 rapid guided repetitions. Pairwise distances (p-distance) were calculated in MEGA 6 using the uncorrected p-distance model.

Results

Morphological comparisons

The morphological comparisons of *Pelophylax plancyi* and *P. chosenicus* revealed similarities, our newly-collected specimens of *P. plancyi* (P) and *P. chosenicus* (C) both had large body sizes (SVL: 40.7–56.1 mm, n = 6 (P) vs. 41.1–60.1 mm, n = 6 (C)), head length slightly larger than head width (HL/HW: 1.00–1.03, n = 6 (P) vs. 1.01–1.04, n = 6 (C)), relatively short snouts (SL/SVL: 0.14–0.16, n = 6 (P) vs. 0.14–0.16, n = 6 (C)), tympanum diameter slightly smaller than the eye diameter (ED/TD: 1.02–1.09, n = 6 (P) vs. 1.00–1.15, n = 6 (C)), internal subgular vocal sacs and dorsolateral fold slightly thick. Furthermore, all of the newly-collected *P. hubeiensis* specimens can be distinguished from *P. plancyi* and *P. chosenicus* by the combination of the following characteristics: (1) head width slightly larger than head length, HL/HW 0.93–0.97 (n = 6) (vs. head length slightly larger than head width in *P. plancyi* and *P. chosenicus*, HL/HW 1.00–1.04, n = 12); (2) tympanum diameter slightly larger than the eye diameter ED/TD 0.86–0.97 (n = 6) (vs. tympanum diameter slightly smaller than the eye diameter, ED/TD 1.00–1.15, n = 12); (3) foot length shorter, FL/SVL 0.49–0.58 (n = 6) (vs. FL/SVL 0.55–0.78, n = 12); (4) silent sac (vs. internal subgular vocal sacs) (Table 2). The differences between *P. plancyi*, *P. chosenicus*, *P. hubeiensis* and other congeneric species distributed in China are summarised in Table 3.

Moreover, the PCA result indicated that the *Pelophylax hubeiensis* could be distinctly separated from *P. plancyi* and *P. chosenicus*, but overlapped between *P. plancyi* and *P. chosenicus* (Fig. 2). The first two extracted principal component axes accounted for 74.11% and 15.74% of the variation (Table 4). PC1 separated the sexes and the

Table 2. Measurements of adult specimens of *P. plancyi*, *P. chosenicus* and *P. hubeiensis* (# topotype of the three species).

Species	*P. plancyi*						*P. chosenicus*						*P. hubeiensis*					
Specimen	SYAU BAA000035#	SYAU BAA000072#	SYAU BAA000077	SYAU BAA000073#	SYAU BAA000074	SYAU BAA000075	HGSE 01 #	SYAU BAA000046	SYAU BAA000061	HGSE 02 #	SYAU BAA000016	SYAU BAA000062	SYAU BAA000052 #	SYAU BAA000049	SYAU BAA000050	SYAU BAA000053 #	SYAU BAA000051	SYAU BAA000048
Sex	♂	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♀
SVL	40.7	40.9	41.7	55.2	57.2	56.1	41.1	42.0	41.3	56.2	58.8	60.1	41.8	42.0	43.3	55.5	55.9	56.6
HL	16.1	15.9	16.2	23.1	20.5	20.2	15.9	16.1	16.0	21.1	20.5	24.3	16.0	16.1	16.3	18.5	18.6	19.2
HW	16.0	15.8	15.8	22.6	20.0	20.0	15.6	15.7	15.7	20.6	20.3	23.3	17.2	17.3	17.5	19.4	19.6	19.8
SL	6.0	5.9	5.8	8.9	8.0	9.1	6.5	6.0	5.9	7.8	8.5	8.4	5.6	5.8	6.0	6.6	6.9	7.0
INS	3.0	2.9	3.2	3.9	3.5	3.9	3.4	3.3	3.1	3.9	3.5	4.3	3.7	4.1	3.8	3.6	3.6	3.8
IOS	2.9	2.8	2.8	3.7	2.9	3.7	3.3	3.0	2.9	3.6	2.8	4.0	2.1	2.2	2.2	2.5	2.6	2.7
ED	4.7	4.9	4.5	6.5	5.8	6.0	4.7	4.7	4.8	5.3	6.2	6.6	5.1	5.2	5.4	6.0	5.8	6.2
LAHL	18.1	18.3	19.6	26.9	24.9	27.1	18.0	18.7	19.0	24.3	22.5	27.5	18.5	18.6	19.0	24.1	25.0	25.3
TD	4.6	4.5	4.6	6.4	5.4	5.5	4.7	4.6	4.5	5.2	5.4	6.2	5.9	5.9	6.1	6.2	6.0	6.5
LAD	4.7	4.7	4.9	5.5	5.9	5.8	4.8	5.0	4.8	5.2	4.6	5.6	3.9	4.2	4.0	4.8	5.0	5.1
HLL	61.3	62.5	62.7	88.2	84.8	85.1	62.5	64.0	62.6	85.4	86.6	89.5	66.0	66.2	66.5	77.2	78.5	78.3
TL	17.7	18.1	19.0	27.5	25.7	24.9	19.2	18.5	18.2	24.7	23.3	28.5	18.4	18.2	18.7	25.1	25.5	25.5
TW	5.5	5.7	5.6	7.5	7.2	7.6	5.9	5.8	5.3	7.2	8.4	7.7	6.2	6.3	6.6	7.5	7.8	8.2
FL	31.1	32.0	31.0	39.8	39.1	38.0	29.4	32.1	31.5	32.9	32.1	43.0	24.2	24.2	24.5	28.8	28.6	27.8
IMT	3.0	3.1	3.2	4.9	5.1	5.0	3.3	3.2	3.2	4.8	5.1	5.0	3.2	3.1	3.3	4.8	4.9	4.7

Table 3. Brief morphological comparisons amongst *Pelophylax* species distributed in China.

Species	Head style	Vocal sac	Web	Background colouration	Dorsolateral fold	Reference
P. plancyi	HL slightly larger than HW	internal subgular vocal sacs	almost full web, webbing formula I 0-0⁻ II 0-⅓ III 0 – ⅓ IV I ⅓ – 0 V	green or olive green	slightly thick	Fei et al. (2009)
P. chosenicus (sensu *P. plancyi*)	HL slightly larger than HW	internal subgular vocal sacs	almost full web	green, olive green or emerald green	slightly thick	Zhou et al. (2022)
P. hubeiensis	HW slightly larger than HL	silent sac	almost full web, webbing formula I 0⁻ – ⅓ II 0⁻-⅓ III ⅓ – I⁺ IV I⁺ – 0⁻ V	green, olive green or light brown mixed with green flecks	thick	Fei et al. (2009)
P. mongolius	HW slightly larger than HL	external lateral vocal sacs	entire web, webbing formula I 0 -1 II ⅓-1½ III 1 – 2 – + IV 2 -1 V	light green with a few black patches, Green to brown gradation or brown	slightly thick	Zhou et al. (2022)
P. nigromaculatus	HL larger than HW	external lateral vocal sacs	the fourth finger webbed up to the distal end of the first subarticular tumor, the rest up to the fingertip, gap deep	light green, chartreuse. Dark green and taupe with irregular dark spots	range from narrow to thick	Fei et al. (2009)
P. fukienensis	HL slightly larger than HW	internal subgular vocal sacs	almost entire web	green or brownish-green, a few individuals with small black spots	narrow	Fei et al. (2009)
P. terentievi	HW and HL almost isometric	external lateral vocal sacs	entire web	olive green with dark brown rounded markings	thick	Fei et al. (2009)

Table 4. Variable loadings for principal components with eigenvalue.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
SVL	-0.28	-0.06	-0.27	0.06	0.10	-0.09	0.02	-0.33	0.06	-0.03
HL	-0.28	0.08	0.15	0.15	-0.30	-0.30	-0.05	-0.14	0.22	-0.27
HW	-0.29	-0.08	0.14	0.08	-0.27	-0.30	-0.09	-0.04	-0.08	-0.19
SL	-0.28	0.15	0.00	0.43	0.13	0.27	-0.24	0.59	-0.12	0.14
INS	-0.20	-0.29	0.68	0.02	0.36	0.24	-0.23	-0.34	0.02	0.07
IOS	-0.18	0.45	0.37	0.17	0.25	-0.15	0.64	0.07	0.00	0.08
ED	-0.27	-0.22	-0.14	0.04	-0.30	0.61	0.42	-0.25	-0.16	0.08
LAHL	-0.29	0.01	-0.06	-0.33	0.14	-0.07	0.05	0.11	-0.72	-0.40
TD	-0.19	-0.46	0.24	-0.38	-0.22	-0.05	0.08	0.53	0.16	0.13
LAD	-0.21	0.39	-0.10	-0.55	0.28	0.27	-0.21	0.00	0.33	-0.18
HLL	-0.29	-0.01	-0.06	0.23	0.01	-0.12	-0.35	-0.07	-0.06	-0.04
TL	-0.29	-0.01	-0.08	-0.29	0.02	-0.32	0.15	-0.05	0.14	0.43
TW	-0.26	-0.24	-0.27	0.21	0.20	0.10	0.22	0.16	0.46	-0.40
FL	-0.20	0.45	0.13	-0.07	-0.55	0.25	-0.16	-0.04	0.06	0.11
IMT	-0.29	-0.03	-0.31	0.04	0.19	-0.11	-0.12	-0.08	-0.11	0.53
variance	0.74	0.16	0.04	0.02	0.01	0.01	0.01	0.01	0.00	0.00

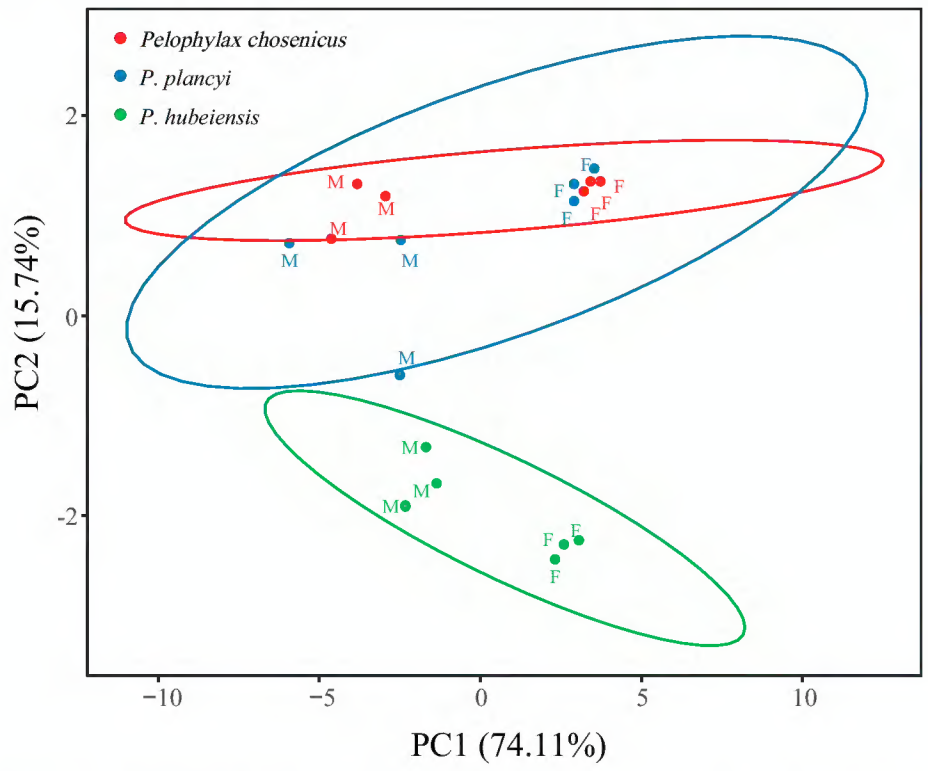

Figure 2. Plots of the first principal component (PC1) versus the second (PC2). *Pelophylax chosenicus* (red), *P. plancyi* (blue) and *P. hubeiensis* (green). M: Male; F: Female.

Interorbital distance, Horizontal tympanic diameter and Foot length are those loading strongly on PC2, were the most important morphological indexes affecting the morphological differences amongst the three species in PCA.

Molecular phylogeny

In this study, the topological structures of the Maximum Likelihood (ML) and Bayesian Inference (BI) trees are generally consistent (Fig. 3; Table 5). The genus *Pelophylax* forms a monophyletic group (Clade A), which is divided into two groups (Clades A1 and A2). In the gene tree, samples of *Pelophylax plancyi* and *P. chosenicus* (including topotype) form a supported monophyletic group (Clade B; Bayesian posterior probabilities 100, bootstrap supports 1.00) and small divergences (p-distance 0.0–0.8), which is further close to the *P. nigromaculatus* with significant support (Bayesian posterior probabilities 96, bootstrap supports 0.98). The samples of *P. hubeiensis* (including a topotype) also form a strongly-supported monophyletic group (Clade C; Bayesian posterior probabilities 100, bootstrap supports 0.99) far from clade B and with moderate divergence (p-distance 1.4–2.0).

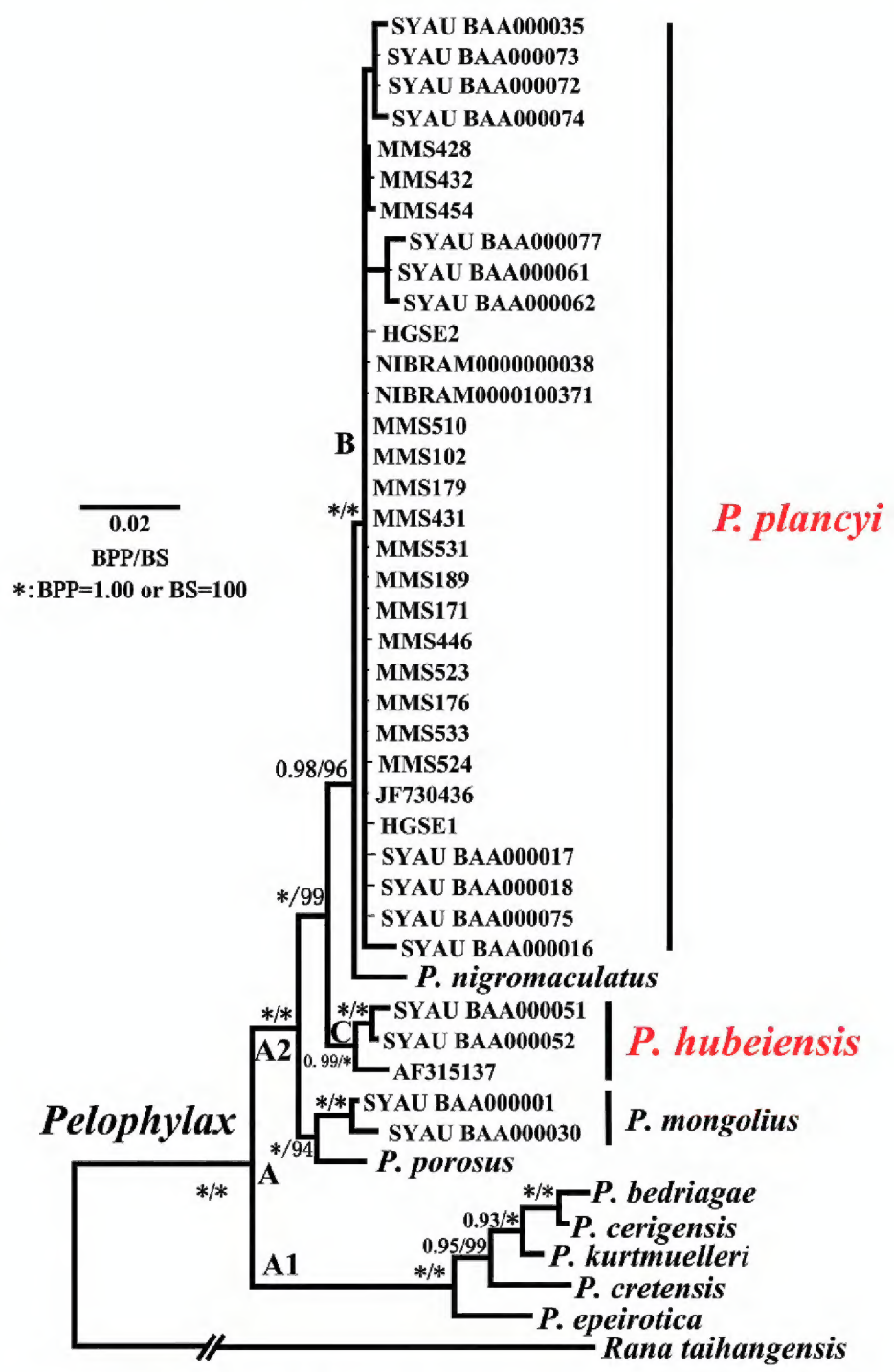

Figure 3. Bayesian-inferenced topology of *Pelophylax* species, based on *16s* rRNA sequences. BPP and BS values, respectively, occur at the nodes.

Taxonomic account

The combined evidence from morphology and molecular phylogeny suggested the specific distinction of *Pelophylax hubeiensis* that is distant from *P. plancyi*, while indicating the homogeneity between *P. plancyi* and *P. chosenicus*. Thus, we suggest that *P. chosenicus* is a junior synonym of *P. plancyi* and *P. hubeiensis* should be treated as a distinct species and we provide descriptions of these two separate species.

Table 5. Uncorrected p-distances (in %) amongst the *Pelophylax* species in this study.

ID	Species	I	II	III	IV	V	VI	VII	IIX	IX	X	XI
I	*Pelophylax plancyi*	0.0–0.5	–	–	–	–	–	–	–	–	–	–
II	*P. chosenicus*	0.0–0.7	0.0–0.5	–	–	–	–	–	–	–	–	–
III	*P. nigromaculatus*	0.9–1.1	0.8–1.1	0.0	–	–	–	–	–	–	–	–
IV	*P. hubeiensis*	1.4–2.0	1.5–1.9	1.0–1.1	0.0–0.7	–	–	–	–	–	–	–
V	*P. porosus*	2.7–3.1	2.6–3.0	2.1	2.5–2.7	0.0	–	–	–	–	–	–
VI	*P. mongolius*	2.4–3.3	2.5–3.1	2.3–2.9	2.7–3.2	2.3–2.5	0.0–0.5	–	–	–	–	–
VII	*P. kurtmuelleri*	7.1–7.3	7.1–7.4	7.3	7.2–7.4	8.2	7.3–7.4	0.0	–	–	–	–
IIX	*P. epeiroticus*	8.0–8.2	8.0–8.2	7.4	7.8–8.3	8.0	7.8–7.8	3.9	0.0	–	–	–
IX	*P. cretensis*	8.9–9.1	9.0–9.1	8.5	8.0–8.2	8.4	8.2–8.3	2.9	4.5	0.0	–	–
X	*P. cerigensis*	7.4–8.0	7.5–8.0	7.4	7.3–7.6	8.1	7.3–7.4	1.3	3.9	3.7	0.0	–
XI	*P. bedriagae*	7.3–8.0	7.3–8.0	7.3	7.4–7.6	7.7	7.3–7.5	1.9	4.3	3.9	0.8	0.0

Pelophylax plancyi (Lataste, 1880)

Rana plancyi – Lataste 1880; Boulenger (1920).
Rana nigromaculata coreana – Okada (1927, 1928).
Rana nigromaculata chosenica – Okada (1931).
Rana chosenica – Kuramoto (1983).
Rana plancyi plancyi – Kuramoto (1983).
Rana (*Rana*) *plancyi* – Dubois (1986).
Rana (*Rana*) *chosenica* – Dubois (1986).
Rana (*Pelophylax*) *plancyi* – Dubois (1992).
Rana (*Pelophyxlax*) *chosenica* – Dubois (1992).
Rana (*Pelophylax*) *plancyi chosenica* – Dubois and Ohler (1994).
Hylarana plancyi – Chen et al. (2005).
Hylarana chosenica – Chen et al. (2005).
Pelophylax chosenicus – Frost et al. (2006); Che et al. (2007); Zhou et al. (2022).

Type series. *Syntypes*: Not traced, two specimens presumably originally in Lataste's personal collection and which were deposited ultimately in the BMNH; BMNH 1920.1.20.1020 is by museum records a syntype (Lataste 1880; Frost 2022). ***Neotype*:** CIB 79I1764, adult male, collected from Yongfeng, Beijing, China (Fei et al. 2009).

Specimens examined. Twelve newly-collected specimens (six adult males and six adult females): adult males SYAU BAA000035, SYAU BAA000072 and adult female SYAU BAA000073, from Nanshan Park, Jiujiang, Jiangxi, China (29.6698°N, 116.0057°E, 28 m a.s.l.) (type locality of *Pelophylax plancyi*); adult male HGSE 01 and adult female HGSE 02 from Liulin Wetland, Seoul, South Korea (37.5663°N, 126.9779°E, 45 m a.s.l.) (type locality of "*P. chosenicus*"); adult male SYAU BAA000046 and adult female SYAU BAA000016 from Puhe Park, Shenyang, Liaoning, China (41.5114°N, 122.7579°E, 30 m a.s.l.); adult male SYAU BAA000061 and adult female SYAU BAA000062 from Donggang District, Dandong, Liaoning, China (39.8260°N, 124.0526°E, 60 m a.s.l.); adult female SYAU BAA000074 from Yuzui Park, Nanjing, Jiangsu, China (31.9801°N, 118.6723°E, 26 m a.s.l.); adult female SYAU BAA000075 from Bailuhu Park, Binzhou, Shandong, China (37.4209°N, 118.1575°E, 55 m a.s.l.); adult male SYAU BAA000077 from Si River, Jining, Shandong, China (35.5338°N, 116.8349°E, 45 m a.s.l.).

Diagnosis. (1) large body size, SVL 40.7–59.6 mm in adult males (n = 16), SVL 55.2–70.5 mm in adult females (n = 16) (Fei et al. 2009); (2) head length slightly larger than head width; (3) maxillary gland pronounced; (4) tympanum diameter large, slightly smaller than the eye diameter; (5) loreal region oblique, concave; (6) nostril located dorsally, between the eye and the tip of snout; (7) vomerine teeth two small masses; (8) tongue pyriform, free-margin notched; (9) fingers with rudimentary webs; toes almost full webs, webbing formula I 0 -0⁻ II 0-⅓ III 0 – ⅓ IV I ⅓ – 0 V; (10) heels only meeting.

Common names. "Green Pond Frog" in English / "金线侧褶蛙 (jīn xiàn cè zhě wā)" in Chinese.

Comparison. *Pelophylax plancyi* can be differentiated from other species in the *Pelophylax nigromaculatus* species group (*P. nigromaculatus*, *P. terentievi* and *P. mongolius*), based on the internal subgular vocal sacs in males (Fei et al. 2009). *Pelophylax plancyi* additionally differs from *P. hubeiensis*, based on its head slightly longer than wide, its tympanum slightly smaller than the eye diameter and longer foot (see Morphological Comparisons in the Results above). *Pelophylax plancyi* further differs from *P. fukienensis*, based on the dorsolateral fold wider and heels meeting (vs. dorsolateral fold narrower and heels meeting in *P. fukienensis*).

Colouration. The living specimens exhibit varying degrees of green, olive green or emerald green body colour variation, without spots on their backs, a tympanic membrane that was golden yellow or brown with a green margin and yellow eyelids. The dorsolateral fold ranged from green to golden yellow or light brown, with yellow at the ends and some minuscule irregular yellow spots. The legs were slightly lighter than the body, with brownish-yellow transverse stripes. The throat, chest and belly were light yellow. The backs of the elbows were yellow, with light brown cloudy spots behind the thighs. The ventral surface of the forelimbs and hind limbs were yellow. The nuptial pads were light grey (Fig. 4A, B).

In preservative, the dorsal surface turned dark grey without patches, while the dorsolateral fold and backline were light grey, the limbs were brown with black stripes, the ventral surface was flesh-coloured, the ventral surface of the limb was beige and the hand and toe webs were dark grey (Fig. 5).

Sexual dimorphism. Male with a pair of internal subgular vocal sacs; in the breeding season, a single, light grey nuptial pad on the dorsal surface of finger I. Males slightly smaller than females with linea masculina.

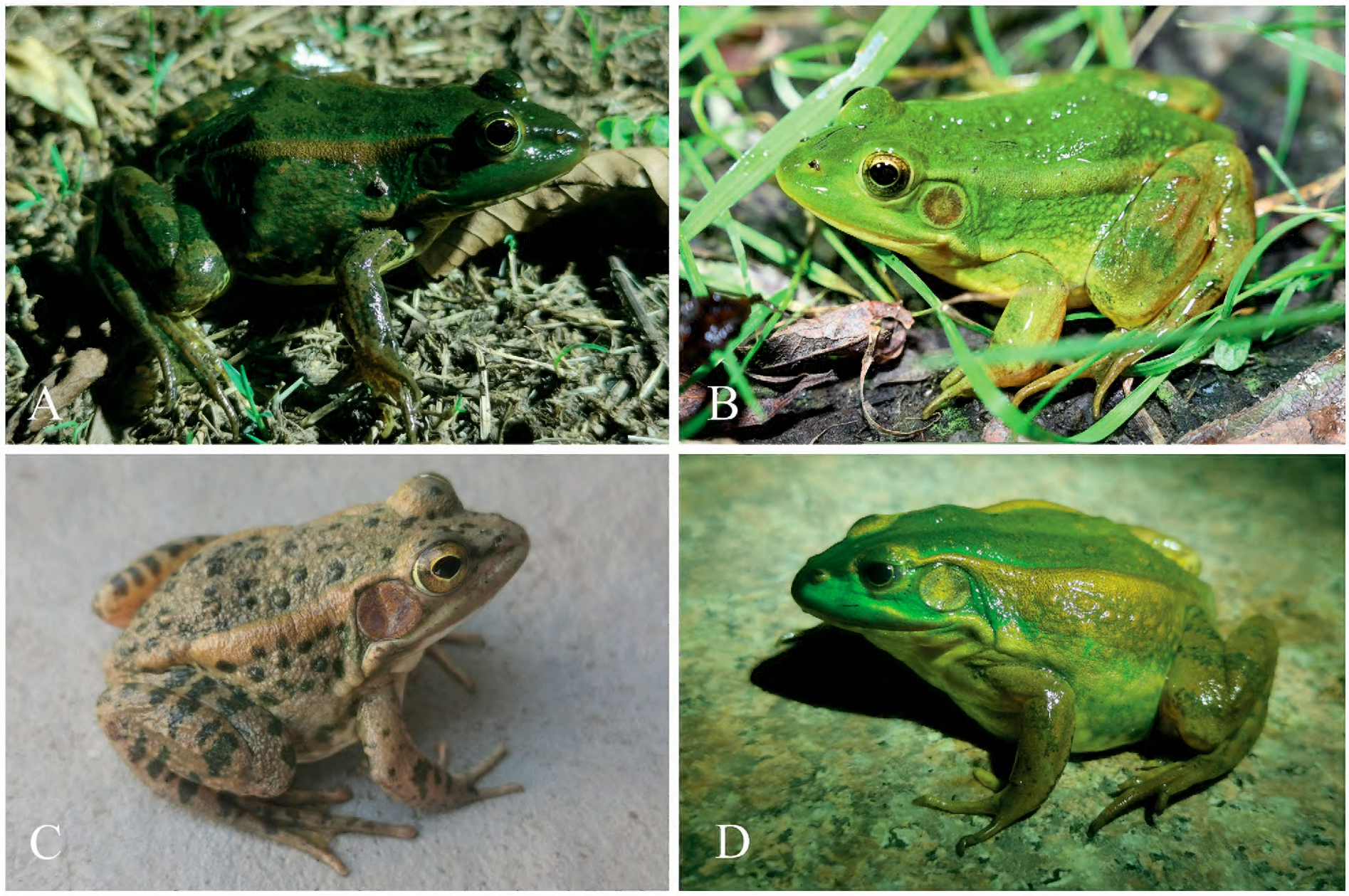

Figure 4. Colouration of *Pelophylax plancyi* **A.** ♀ SYAU BAA000073; **B.** ♂ SYAU BAA000061 and *P. hubeiensis*; **C.** ♂ SYAU BAA000052; **D.** ♀ SYAU BAA000051.

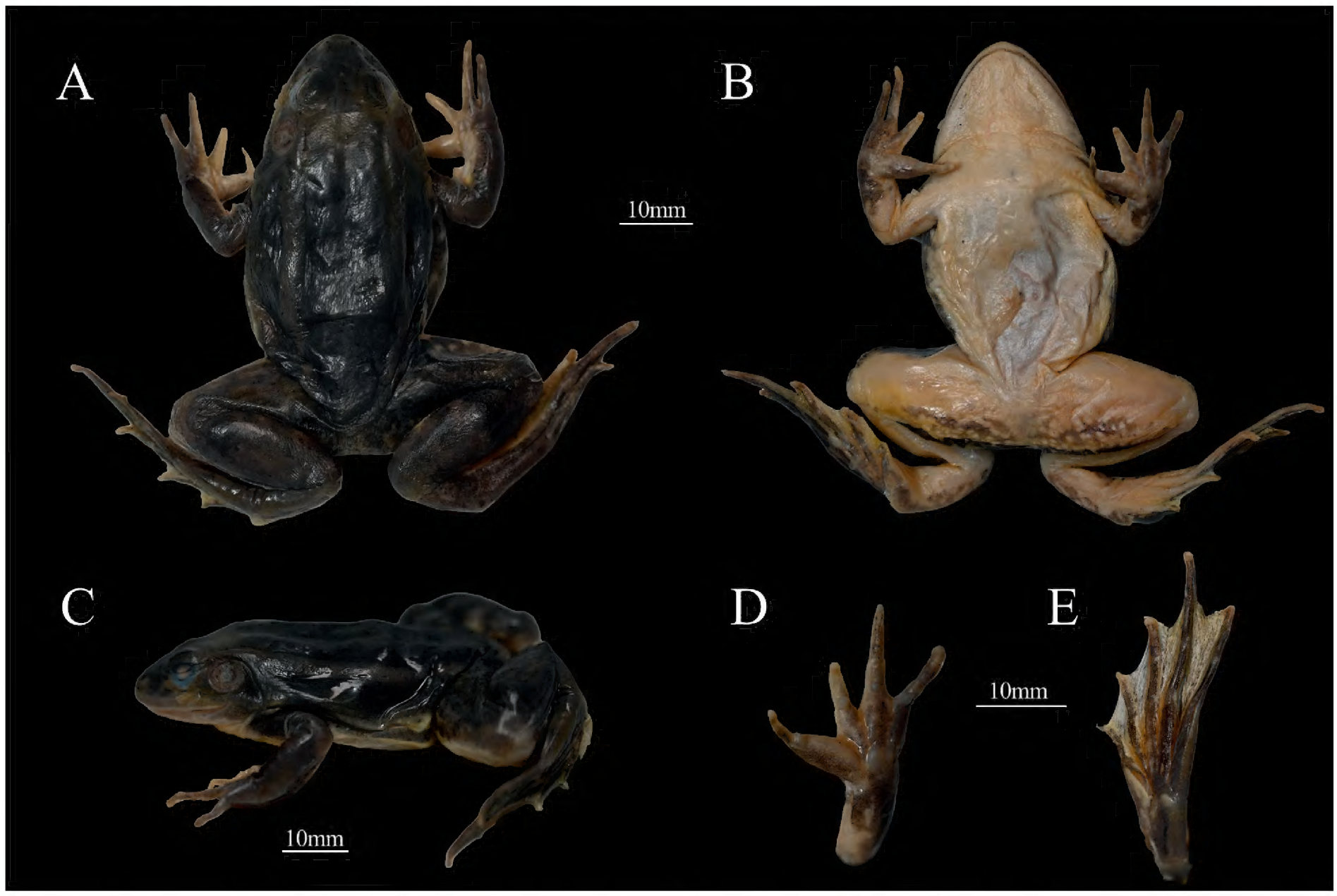

Figure 5. Specimen (SYAU BAA000073) of *Pelophylax plancyi*. **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view; **D.** Ventral view of the hand; **E.** Ventral view of the foot.

Distribution and ecology. At present, specimens of *Pelophylax plancyi* have been identified in eastern China (except Hunan, Guangdong, Jilin and Heilongjiang) and the Korean Peninsula (Fei et al. 2009; Zhou et al. 2022). Specimens were collected from ponds full of aquatic plants (Fig. 7A, B), where they perch along the river bank during the night-time, although, when startled, they jump into the water.

Pelophylax hubeiensis (Fei & Ye, 1982)

Rana hubeiensis – Fei and Ye (1982).
Hylarana hubeiensis – Chen et al. (2005).
Pelophylax plancyi – Dufresnes and Litvinchuk (2022).

Type series. ***Holotype***: CIB 74I0570, adult male, SVL 43.7 mm, collected from Lichuan, Hubei, China (Fei and Ye 1982).

Specimens examined. Six newly-collected specimens (three adult males and three adult females): adult male SYAU BAA000052 and adult female SYAU BAA000053, from Qing river, Lichuan, Hubei, China (30.3465°N, 108.9988°E, 780 m a.s.l.) (type locality of *Pelophylax hubeiensis*); adult males SYAU BAA000049, SYAU BAA000050 and adult females SYAU BAA000048, SYAU BAA000051 from Jiefang Paek, Wuhan, Hubei, China (30.6141°N, 114.3045°E, 40 m a.s.l.).

Diagnosis. (1) large body size, males slightly smaller SVL 38.5–47.1 mm in adult males (n = 23), SVL 41.1–61.9 mm in adult females (n = 23) (Fei et al. 2009); (2) head width slightly larger than head length; (3) maxillary gland pronounced; (4) tympanum diameter large, slightly larger than the eye diameter; (5) loreal region oblique, concave; (6) nostril located dorsally, between the eye and the tip of snout; (7) vomerine teeth two small masses; (8) tongue long pyriform, free-margin notched; (9) fingers with rudimentary webs; toes almost full webs, webbing formula I 0^{-} – ⅓ II 0^{-} -⅓ III ⅓ – I^{+} IV I^{+} – 0^{-} V; (10) heels not meeting.

Common names. "Hubei Gold-striped Pond Frog" in English / "湖北侧褶蛙 (hú běi cè zhě wā)" in Chinese.

Comparison. *Pelophylax hubeiensis* can be differentiated from all species in the *Pelophylax* genus, based on the silent sac in males (Fei et al. 2009).

Colouration. The living specimens exhibit green, olive green or light brown body colour variation, with some individuals exhibiting green flecks on their backs that were absent on other individuals, a tympanic membrane that was golden yellow or light brown and yellow eyelids. The dorsolateral fold ranged from yellow to light brown or golden yellow. The legs exhibit yellow, brown or light brown colour variation, with green or olive green markings. The throat, chest and belly were light yellow. The backs of the elbows were dark brown, with brown cloud spots behind the thighs. The ventral surface of the forelimbs and hind limbs were yellow. The nuptial pads were grey (Fig. 4C, D).

In preservative, the dorsal surface turned dark olive green with light black patches, while the dorsolateral fold and backline were brownish-yellow, the limbs were light brown with dark brown patches, the ventral surface was beige with black stripe patterns, the ventral surface of the limb was light yellow and the hand and toe webs were beige (Fig. 6).

Sexual dimorphism. Males with a single, grey nuptial pad on the dorsal surface of finger I in the breeding season. Males slightly smaller than females with linea masculina.

Distribution and ecology. At present, specimens of *Pelophylax hubeiensis* have been identified in Henan, Hubei, Anhui, Hunan, Chongqing and Jiangxi, China (Fei et al. 2009). Specimens were collected from ponds with lotus flowers or aquatic plants (Fig. 7C, D), where they perch on the leaves of the aquatic plants during the night-time, although, when startled, they plunge underwater.

Discussion

Given the fact that the *Pelophylax plancyi* species group (*P. plancyi*, *P. fukienensis*, *P. hubeiensis* and *P. chosenicus*) is widely distributed in eastern China and the Korean Peninsula, there are high levels of interspecific morphological and molecular similarity that make the division of species more challenging (Fei et al. 2009). In this study, following the integrative concepts of "morphospecies" and "phylospecies" (Zhou et al. 2022), both molecular and morphological data suggest that *P. plancyi* and *P. chosenicus* are the same species, but *P. hubeiensis* is a distinct species. Based on the priority of names designated by the International Code of Zoological Nomenclature (ICZN), *P. chosenicus* should be considered a junior synonym of *P. plancyi*.

Some researchers have found introgression amongst species within the genus *Pelophylax*, especially between *P. plancyi* and *P. nigromaculatus* (Zhang et al. 2008; Komaki et al. 2015). Therefore, mitochondrial introgression may render such evidence inconclusive, but comprehensive analysis of other aspects of evidence can supplement this deficiency. Although the sequencing of *Pelophyllax* nuclear genes was very difficult, some researchers had overcome these difficulties to obtain nuclear genes sequences. The nuclear analyses of E-Asian *Pelophylax* evidence have shown the homogeneity of *P. plancyi* and *P. chosenicus* (Komaki et al. 2015), which was consistent with the viewpoint of this article. Although there was introgression between *P. plancyi* and *P. hubeiensis*, the genetic distance between them was still far enough to support their separation into two species. The above viewpoints were supported by morphological data. The morphological differences between females and males are greater than those between species.

The acquisition of nuclear genes sequencing in *Pelophylax* genus will provide favourable evidence for further verification of species relationships and researchers look forward to breaking this barrier as soon as possible. We will also continue to search for new evidence (such as ecological characteristics, behavioural characteristics and developmental characteristics) as a supplement to molecular and morphological data.

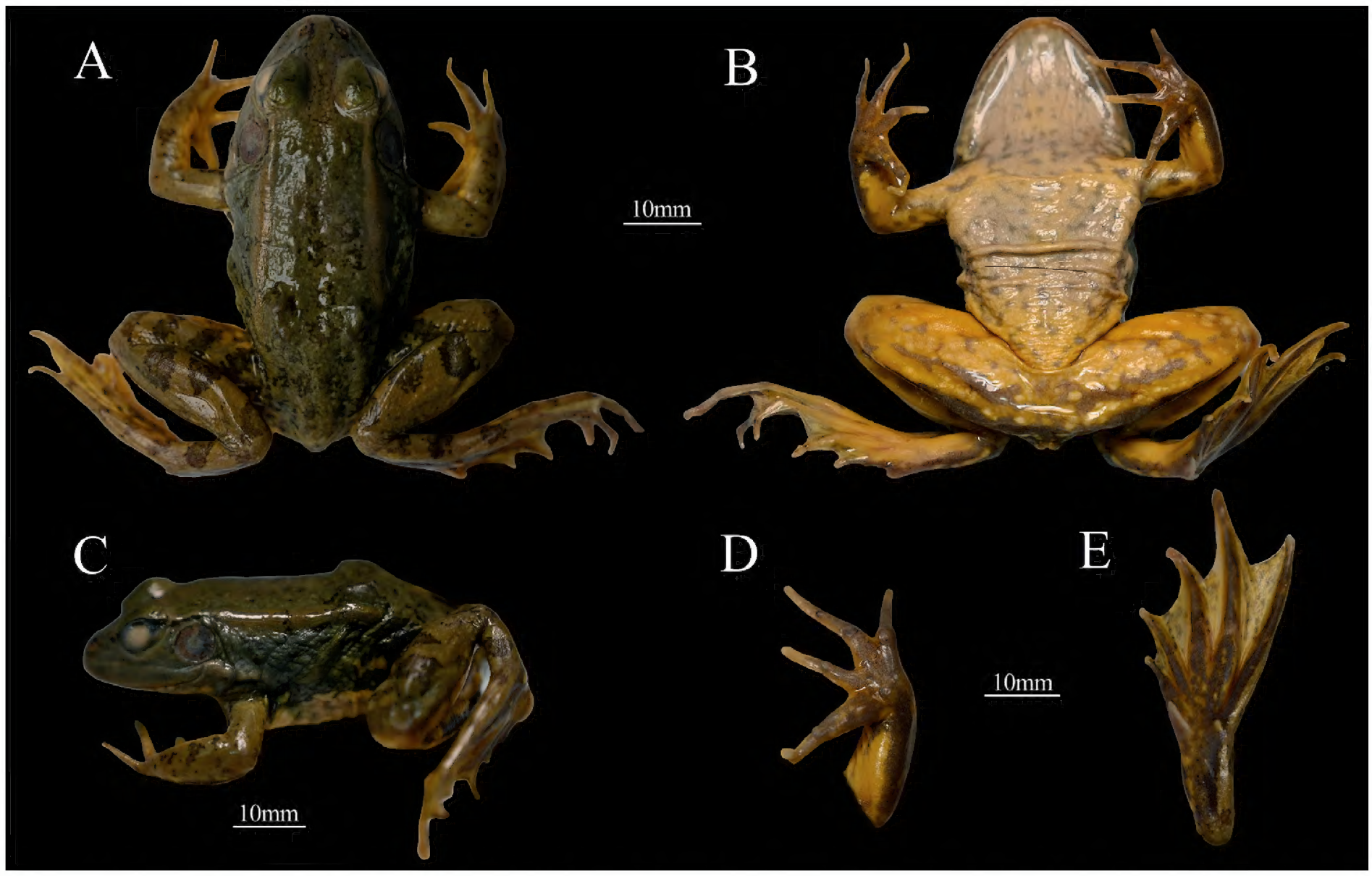

Figure 6. Specimen (SYAU BAA000053) of *Pelophylax hubeiensis*. **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view; **D.** Ventral view of the hand; **E.** Ventral view of the foot.

Figure 7. The habitat of *Pelophylax plancyi* (**A**) macrohabitats, (**B**) microhabitats and *P. hubeiensis* (**C, D**).

The shortcomings of taxonomy also exist in the species of *Pelophylax* genus. Given the fact that *P. plancyi* and *P. hubeiensis* are sympatric species in eastern-central China (Zhao et al. 2009), further studies and molecular data are needed to determine whether there is introgression or if they produce filial generations between the two species. The direction and timing of mitochondrial gene infiltration had not been elucidated in existing studies and research on this issue is not yet sufficiently in-depth. More species need to be applied for detailed elucidation.

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